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Technique

A comparison of stable and ^{14}C-labelled polyethylene glycol as volume indicators in the human jejunum[1]

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SUMMARY Polyethylene glycol (PEG plus ^{14}C-PEG) was measured turbidimetrically and by liquid scintillation counting to compare the validities of these methods during the use of PEG as a volume indicator of intestinal perfusion studies in man. Use of ^{14}C-PEG results yielded similar estimates of water absorption or secretion. The simplicity of ^{14}C counting offers practical advantages to the use of ^{14}C-PEG as a nonabsorbable marker for perfusion studies in man.

Intestinal perfusion, utilizing 'nonabsorbable' volume indicators, is used widely as a technique for assessing absorption in intact man (Soergel, 1971). Polyethylene glycol (PEG) is the accepted standard marker substance because absorption is not significant (Miller and Schedl, 1970) and recovery from the human gut is complete (Malawer and Powell, 1967; Shields, Harris, and Davies, 1968). Perfusion studies rely on sensitive, accurate, and reproducible assay of marker concentrations, yet the usual turbidimetric analysis of PEG (Hydén, 1956) is relatively insensitive and may be inaccurate after thawing of samples frozen for prolonged periods (Miller and Schedl, 1970). Several analytical modifications have been proposed (Malawer and Powell, 1967; Boulter and McMichael, 1970), but the methods are laborious. A more convenient compound, ^{14}C-labelled PEG, has been studied in a rat model (Miller and Schedl, 1970).

We report here a comparison in man of ^{14}C-labelled and stable PEG made during studies in which considerable variations in net water movement were induced.

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Methods

Fasting, healthy human volunteers, who had given informed consent, swallowed a four-lumen tube that incorporated an occlusive balloon (Phillips and Summerskill, 1966). Under fluoroscopic control, the tube was allowed to progress until the balloon was at the ligament of Treitz. The balloon was then inflated with 35 ml of air, and duodenal content was aspirated continuously. The perfusion segment was 25 cm of jejunum distal to the balloon; solutions were perfused proximally (Beckman solution, metering pump) at 10 ml/min and sampled by siphonage distally.

Four consecutive experiments, each lasting up to 90 minutes and employing a different solution, were undertaken in each subject. Samples were collected from the bowel in 10-minute aliquots until a steady state was achieved. The PEG concentrations in the last four collections were used to compute mean changes in volumes, relative to the infusate. Statistical comparisons were carried out by least-squares regression analysis or the paired Student t test.

All perfusion fluids had the following composition (m-equiv/l): Na 140, K 5, Cl 105, and HCO_3 40, with glucose 2 g/l. The PEG (Carbowax 4000, Union Carbide Corp., mol wt approximately 4 000, 5 g/l) was added to all, and ^{14}C-PEG (New England Nuclear Corp.) was added to give a concentration of 5 μc/l in two subjects and 10 μc/l in three subjects. The preparation of ^{14}C-PEG is uniformly labelled and has the same average molecular weight (4 000) as the stable form (Mekhjian, Phillips, and Hofmann, 1971). In addition, some perfusion fluids contained known physiological (2·5 to 10 mM/l) concentrations of highly purified conjugated bile acids that induced profound changes in net water transport.

After the collection of each 10-minute sample of perfusate, aliquots were taken for chemical assay and radioassay of PEG after overnight storage at -10 C. A 0·5-ml aliquot was diluted (1:20) with normal saline, and the chemical concentration of PEG was estimated turbidimetrically (Hydén, 1956). Hydén's method was modified further by using double volumes of precipitating agent and filtering immediately; after filtration and addition of trichloracetic acid and barium chloride, samples were allowed to stand for 20 minutes before spectrophotometry at 650 mμ. The coefficient of variation of

this method in our laboratory is 1·4% (Devroede and Phillips, 1969). A 0·2-ml aliquot of the perfusate was added to 15 ml of a dioxane-based scintillation 'cocktail', and counted for ^{14}C (Picker Liquimat or Beckman LS-250). The efficiencies of counting were 87·6% ± 2·6 and 91·6% ± 1·4, respectively, as assessed by external standardization of increasingly quenched ^{14}C standards.

Results

Mean water movements for each experiment in each subject were obtained, and the results with ^{14}C and stable PEG were compared (Table I). In eight of 20 perfusions, the means differed significantly yet no systematic difference was detected, there being four positive and four negative differences. However, these comparisons can be misleading because small differences between values with small standard errors may be of statistical significance but not of practical significance. The ^{14}C and stable PEG analyses always agreed as to whether 'absorption' or 'secretion' occurred, except in one perfusion employing the lower concentration of isotope. Exact quantitative comparisons were less reliable.

Results from all perfusion periods in each subject were pooled, including equilibration periods between different solutions (Table II). A high correlation was found in data from four subjects, three in whom water movement varied widely. In the fifth, the

Subject	*Perfusion Fluid*	*Mean (± SE) Change*[1] *(Chemical Assay)*	*Mean (± SE) Change*[2] *(^{14}C Counting)*	*Mean Difference*
^{14}C concentration in perfusate (5 μc/l)				
	Control	1·34 ± 0·32	0·54 ± 0·14	+0·80
	B1	0·75 ± 0·12	0·43 ± 0·20	+0·33[3]
D	B2	−0·47 ± 0·13	−0·02 ± 0·07	−0·45[5]
	B3	−1·59 ± 0·03	−1·99 ± 0·09	+0·40[3]
	Control	2·07 ± 0·15	1·40 ± 0·99	+0·67
	B1	0·57 ± 0·11	−0·18 ± 0·17	+0·75[4]
R	B2	0·65 ± 0·18	0·89 ± 0·17	−0·24
	B3	−1·45 ± 0·05	−2·52 ± 0·25	+1·07[4]
^{14}C concentration in perfusate (10 μc/l)				
	Control	2·04 ± 0·16	1·99 ± 0·22	+0·05
	B1	1·48 ± 0·45	1·99 ± 0·59	−0·51
H1	B2	1·70 ± 0·06	3·16 ± 0·16	−1·46[6]
	B3	1·29 ± 0·30	1·53 ± 0·30	−0·24
	Control	0·89 ± 0·09	1·09 ± 0·14	−0·20
	B1	0·72 ± 0·26	0·99 ± 0·22	−0·28
H2	B2	−1·32 ± 0·10	−1·24 ± 0·14	−0·07
	B3	−3·24 ± 0·69	−3·81 ± 0·92	+0·56
	Control	2·05 ± 0·07	2·42 ± 0·09	−0·38
	B1	2·35 ± 0·11	2·70 ± 0·05	−0·35[4]
K	B2	1·73 ± 0·11	1·97 ± 0·22	−0·25
	B3	1·40 ± 0·08	1·86 ± 0·12	−0·46[6]

Table I *Differences in mean steady-state volume changes (ml/min) in each of four perfusion studies in five subjects as determined by chemical and isotopic methods*

[1]Each mean volume change is the mean value of four steady-state volume changes, determined from four consecutive 10-minute samples. B1, B2, B3 = bile salt solutions of 2·5, 5·0, 10·0 mM/l, respectively.
[2]Positive volume changes denote 'absorption', and negative volume changes denote 'secretion'. Statistical significance: [3]$P < 0·05$; [4]$P < 0·02$; [5]$P < 0·01$; [6]$P < 0·001$.

	Subject and (Number of samples)				
	D (32)	*R (29)*	*H1 (30)*	*H2 (30)*	*K (26)*
Highest value of x	2·11	2·34	2·43	1·19	2·64
Lowest value of x	−1·64	−1·28	0·45	−4·16	1·17
Highest value of y	1·83	2·42	3·57	1·64	2·88
Lowest value of y	−2·56	−3·45	−0·57	−5·29	1·32
Regression coefficient (b)	0·8812	1·2374	0·7149	1·1391	0·9723
SE of b	0·068	0·1205	0·2808	0·0558	0·0907
Intercept on y axis (a)	−0·1721	−0·6584	0·9202	0·0893	0·3574
Correlation coefficient (r)	0·9211[5]	0·8923[5]	0·4336[1]	0·9680[5]	0·9095[5]

Table II *Characteristics of calculated linear regression ($y = bx + a$) for volume changes (as ml/min) determined by chemical assay (x axis) and volumes determined by counting ^{14}C (y axis)*

Statistical significance: [1]$P < 0·05$; [5]$P < 0·001$.

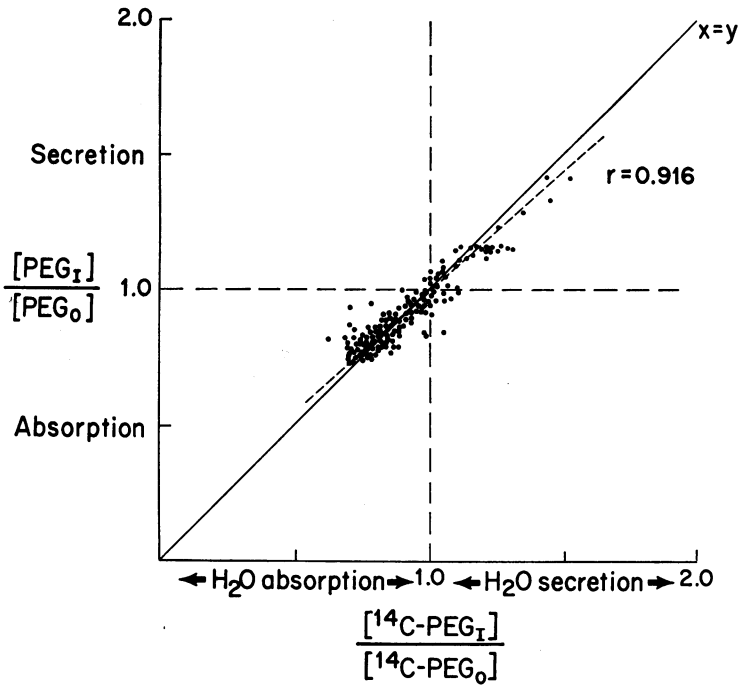

Fig. *Linear regression of changes in volume during jejunal perfusion in 148 separate samples from five human subjects. Results are plotted as marker concentration in/marker concentration out for chemical assay (y axis) and ^{14}C measurement (x axis).*

correlation between chemical and ^{14}C-PEG was of marginal significance owing mainly to the effect of one perfusion period. Similar pooling of all results (n = 148) and expression of each result as concentration marker in/concentration marker out (Ivey and Schedl, 1970) yield a highly significant correlation (Figure). In only eight of the 148 sets of determinations did simultaneous measurement of two markers differ as to whether absorption or secretion occurred. In seven of these samples, changes in concentrations of both markers were 10% or less.

Comment

With our two-lumen perfusion system, volume change (ΔV) is calculated from the volume infused (V_1), the concentration of volume indicator in the infusate (C_1), and the marker concentration in the fluid sample recovered (C_2), using the relationship:

$$\Delta V = V_1 (1 - C_1/C_2).$$

The inclusion of a ratio of two experimentally determined values (C_1/C_2) emphasizes the dependence of perfusion studies on accurate assay of a nonabsorbed solute. The techniques may include other inherent disadvantages (Soergel, 1971). Thus, perfusion studies discriminate between 'secretion' and 'absorption' and also can detect changing rates of net fluid movement, but they cannot be regarded, without qualification, as the source of precise quantitative measurements.

There is general agreement that PEG is the most suitable volume indicator for aqueous perfusates (Jacobson, Bondy, Broitman, and Fordtran, 1963; Malawer and Powell, 1967; Miller and Schedl, 1970), although its value in closed-loop studies (Jacobson *et al*, 1963; Miller and Schedl, 1970) and in the nonaqueous phase of intestinal contents (Wiggins and Dawson, 1961) has been questioned. Turbidimetric assay of PEG (Hydén, 1956) is tedious, insensitive, and unreliable under certain conditions. Attempts have been made to improve the chemical assay (Malawer and Powell, 1967; Boulter and McMichael, 1970), but its sensitivity is still uncertain. An accuracy of 1·4% was claimed by Devroede and Phillips (1969) for 25 replicate determinations, and by Boulter and McMichael (1970) for 157 consecutive determinations, although Jacobson and colleagues (1963) reported a standard deviation of 3·7% for 10 replicate determinations. Malawer and Powell (1967) found that all but 3% of duplicate determinations agreed within 2%.

Our data show clearly that volume changes derived from the labelled PEG may be regarded with the same confidence as is attached to changes calculated from the chemical assay, given an adequate isotope concentration. Indeed, there is no way of determining which set of values should be regarded as 'correct', since there is no absolute reference point.

In our studies, the higher concentration of ^{14}C-

PEG (10 μc/l) yielded about 2 000 dpm, with a counting error on a single specimen of 2·2%. Thus, the precision of determinations of labelled PEG approaches that of duplicate, or multiple-replicate, chemical assays. It follows that acceptable precision in the use of the labelled PEG may be found by counting single specimens, whereas the same level of accuracy in the chemical assay requires replicate analysis. As to the ease of assay, the radioassay, involving only two volumetric deliveries, is less laborious and less time consuming.

The choice of volume indicator may be dictated by circumstance, such as the absence of counting equipment or the presence of other ^{14}C sources in the experimental system. But, other things being equal, ^{14}C-PEG seems to be the volume indicator of choice with respect to acceptable reproducibility and simplicity. Since recovery of PEG from the gut is complete and rapid, the radiation energy of ^{14}C low, and the administered dose small, exposure to ionizing radiation is negligible.

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